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Correlating Vial Seal Tightness to Container Closure Integrity at Various Storage Temperatures

Dr. Derek Duncan
Director Product Line
LIGHTHOUSE

Roger Asselta
VP & Sr. Advisor
Genesis Technical Advisors

Presentation Outline

- What happens to CCI during deep cold storage?
- How does vial seal tightness correlate to maintaining CCI?
- What should you do if you have product requiring deep cold storage?

  (live viral vaccines, gene/cell therapy)

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Glass transition temperature T_g

- Only polymers have a glass transition temperature.
- When the polymer is cooled below this temperature, it loses elasticity and becomes hard and brittle, like glass.
- 'Rubbery state' vs. 'glassy state'

If container closure integrity depends on the elasticity of the rubber stopper, one might expect potential sealing issues below T_g of the rubber stopper.

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Identified CCI issues in deep cold storage

Because deep cold storage (-80 °C to cryo) is colder than the T_g of most rubber stopper formulations (-50 to -70 °C), there is an increased risk of losing CCI if:

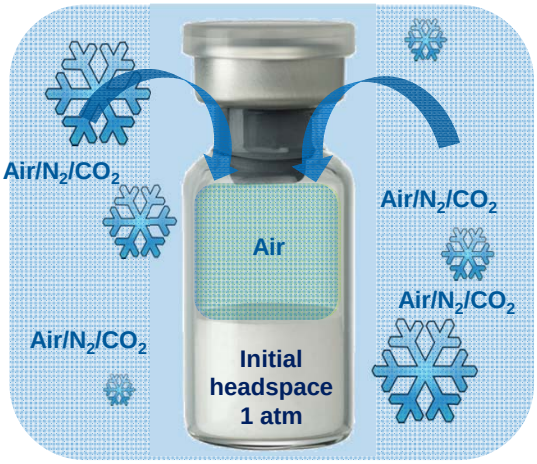
- An appropriate vial / stopper combination is not used
  - Vial / stopper design (blowback, non-blowback, etc.)
  - Vial / stopper fit (relative dimensions stopper plug, inner vial neck)
- Capping / crimping is not done properly
  - 'Tight' vs. 'Loose'

REF: Container/Closure Integrity Testing and the Identification of a Suitable Vial/Stopper Combination for Low-Temperature Storage at -80 C

Brigitte Zuleger, Uwe Werner, Alexander Kort, Rene Glowienka, Engelbert Wehnes, Derek Duncan
PDA J Pharm Sci and Tech 2012, 66 453-465

Leakage in deep cold storage

- Air filled vial at 1 atm at RT
- At low T, initial headspace condenses and creates **underpressure**
- Stopper can lose elastic properties & closure can be lost
- Cold dense gas from storage environment fills headspace
- Warming container to RT, stopper regains elasticity and **reseals**



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Leakage in deep cold storage

- Cold dense gas now trapped inside, expands as temperature goes up, creating **overpressure**
- **Maintenance** of this **overpressure** can be monitored over time to verify that the **leak was temporary**.

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The analytical tool

Rapid non-destructive analytical method for measuring headspace O_2, H_2O, CO_2, and pressure levels

Quantifying the physical headspace conditions with **Laser-based Headspace Analysis** enables identification of CCI dynamics during deep cold storage

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Example: CCI of media filled vials stored on dry ice

- 2R clear tubing vials containing media.
- Initial headspace conditions: 1 atm of air
- Stored on dry ice for 7 days.
- Thawed to room temperature (RT).
- Headspace conditions analyzed.
- Any change in the headspace conditions is a direct sign that closure was lost during deep cold storage resulting in headspace gas exchange.

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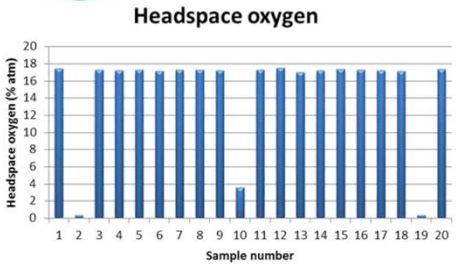
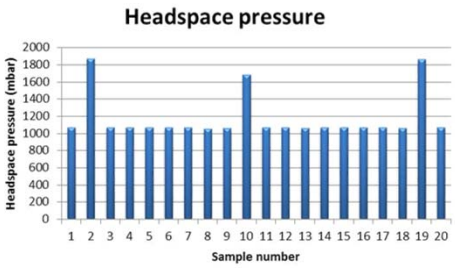
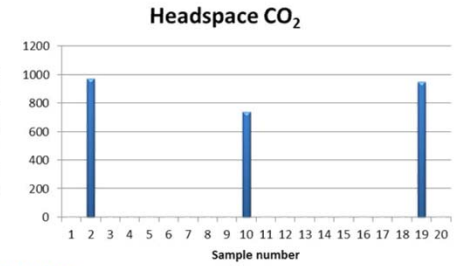
Example: CCI of media filled vials stored on dry ice for 7 days







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Example: CCI of media filled vials stored on dry ice

Some important comments on these results:

- The maintenance of overpressure in the leaking samples over time at RT means the **vial has resealed** at temperatures **above the T_g** of the rubber stopper.
- **Leaks** during deep cold storage are **temporary**!
- **Other CCI methods** (blue dye, microbial ingress, pressure decay) that test these vials after thawing **will NOT identify these vials as having leaked**.

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Elastomeric Closure

- Elastomers are amorphous polymers that exist above their Glass Transition Temperature (Tg) and exhibit viscoelastic behavior (interchangeable with the term rubber).
- Rubber Formulations for closures to seal pharmaceutical containers have Tg s that are usually in the range -50 to -70 ˚C.

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Elastomeric Closure (continued)

- In sealing rubber components, the elastic property is important. An applied stress (sealing force) induces a corresponding strain which creates a contact stress. This stored internal energy is the Residual Seal Force (RSF).
- The viscous property of rubber is also important. It allows considerable segmental motion or flow. This movement can fill gaps and voids in the sealing surface.

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Quantifying vial seal tightness

Residual seal force

- RSF is the stress a compressed elastomeric closure flange continues to exert on a vial land Sealing surface after application of an aluminum seal (crimping)
- Quantifying the RSF is a test method for the indirect estimation of elastomeric closure compression
- Sufficient compression is essential to seal integrity.

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Objectives this cold storage study

- Quantify capping & crimping parameters
  - Three capping pressures defined
  - Measured with Residual Seal Force
- Direct comparison different storage temps
  - Empty capped vials
  - RT, -20˚C, -80 ˚C, and cryogenic (-178 ˚C) storage

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Experimental parameters

- **Stopper/vial combinations**
  - Five different stopper/vial combinations
  - 30 samples of each combi at each temperature and each capping pressure (1800 total)
- **Crimping parameters**
  - Genesis RW-50 capper (rail technology)
  - Crimping pressures (force differentials):
    - Low: 45 N; Med: 98 N; High: 151 N
  - Measured RSF
- **Storage**
  - RT, -20˚C, -80 ˚C, liquid nitrogen

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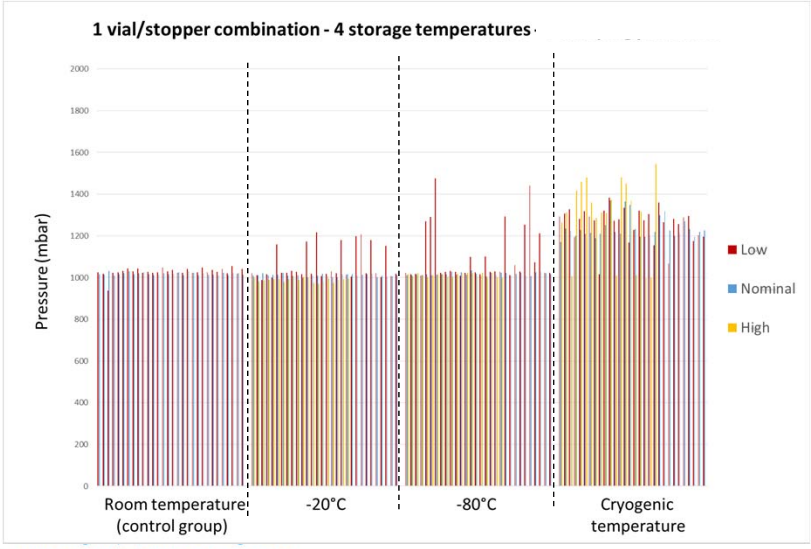
Results: Overpressure vs Temp



Stopper
13mm serum

Vial
2 ml EU BB

3 crimping pressures (RSF)

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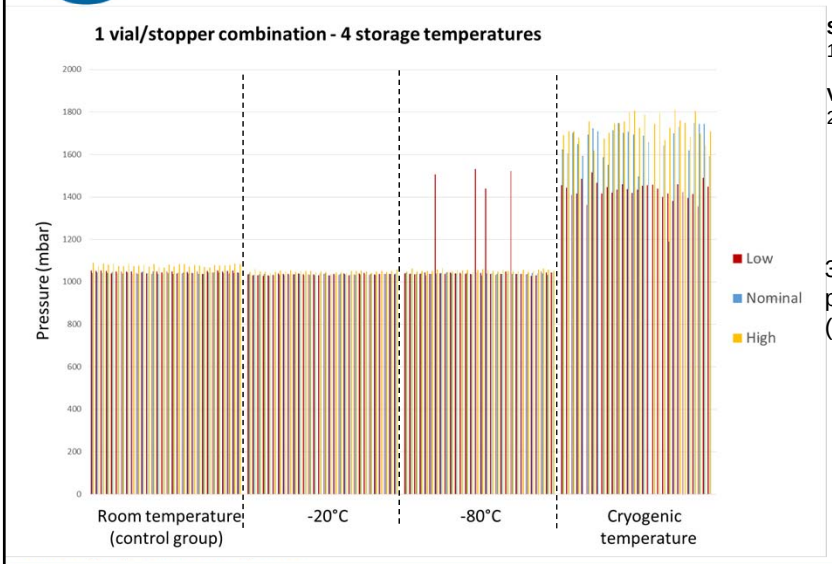
Results: Overpressure vs Temp



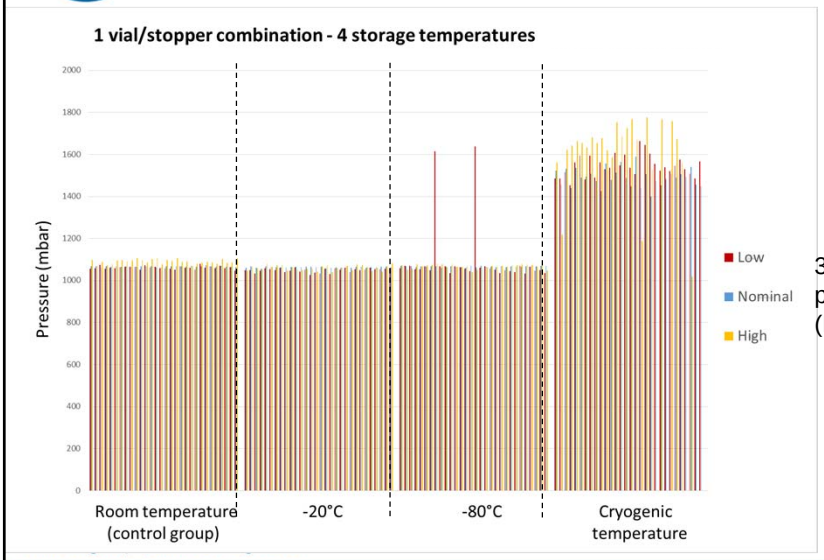
Stopper
13mm Serum

Vial
2 ml EUBB

3 crimping pressures (RSF)

17

Results: Overpressure vs Temp



Stopper
13mm solid plug

Vial
2 ml NonBB

3 crimping pressures (RSF)

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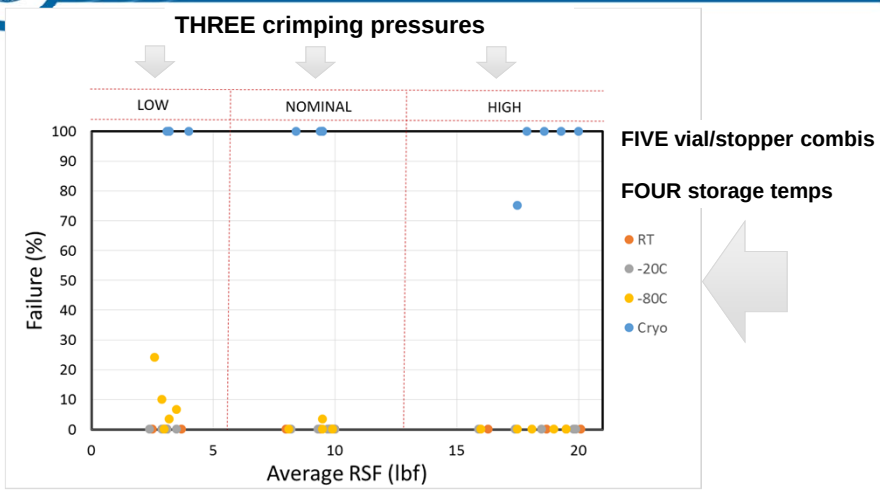
Results: Failure rate vs. RSF



LOW RSF CLEARLY CORRELATES TO HIGH CCI FAILURE DURING DEEP COLD STORAGE

NOTE: Actual avg RSF of low crimped vials is lower, since some RSF were too low to measure (<2lbf)

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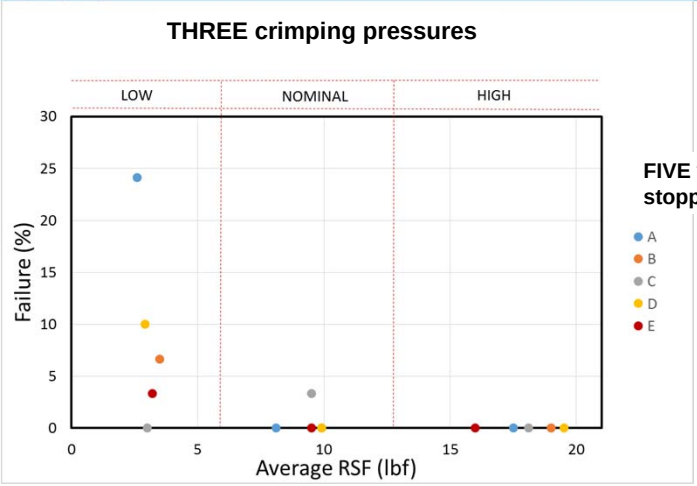
Results: Failure vs. vial / stopper combination at -80 C



Retaining CCI during cold storage is also a function of vial-stopper combination

NOTE: Actual avg RSF of low crimped vials is lower, since some RSF were too low to measure (<2lbf)

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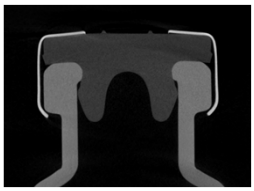
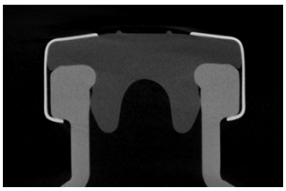
Results: X-ray imaging

Compression Examples Group E



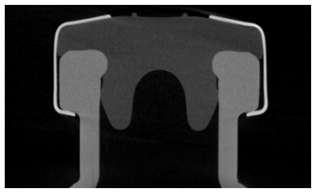
Low crimping pressure

10.3% Compression avg.
3.1 lbs RSF avg



Nominal crimping pressure

22.7% Compression avg.
9.6 lbs RSF avg



High crimping pressure

27.4% Compression avg.
16.5 lbs RSF avg

Images by Micro Photonics Inc. Allentown, PA USA using Bruker Micro CT SkyScan 1173

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Conclusions

- There is **risk for CCI failure** at storage temperatures **below the T_g** of the rubber stopper formulation.
- CCI failures can be mitigated by **ensuring appropriate vial / stopper** combination and **capping & crimping** parameters
- **RSF measurements** can be a useful tool in quantifying seal tightness and predictive of CCI failure at low temps
- **Laser Headspace Analysis** is a suitable non-destructive method to detect (temporary) leaks in cold storage

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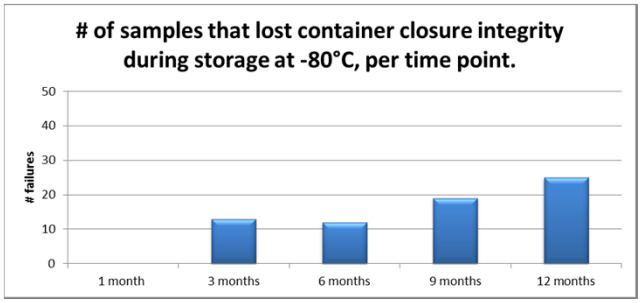
Recent stability results: Failures over storage time

Customer wanted to compare CCI of two stopper vial combinations over 12 months of deep cold storage

- **400 samples of each combination prepared**
- **50 samples taken out of storage and measured at each time point**



- **One combination held CCI over 12 months of storage at -80 ˚C**
- **For other combination, failures started to appear at 3 month time point**

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Alternative container solutions for deep cold storage?

- If rubber stoppers have a $\mathbf{T_g}$ warmer than -80° C, are traditional stoppered vials fundamentally the wrong container to consider for deep cold storage?
- Are there alternative container solutions?

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Alternate container: Crystal® Closed Vials

- Supplied by Aseptic Technologies
- Study performed with **cryogenic storage temp**:
  - 1ml and 6ml Crystal® Closed Vials
  - 13mm stoppered tubing vial (semi-automatic crimper targeting > 30% stopper compression)
  - 20mm stoppered molded vials (hand-crimped)

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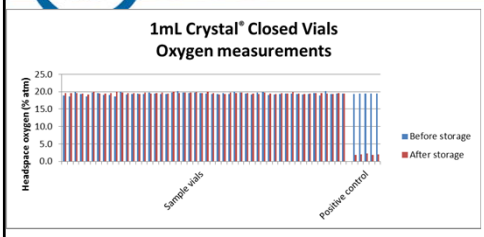
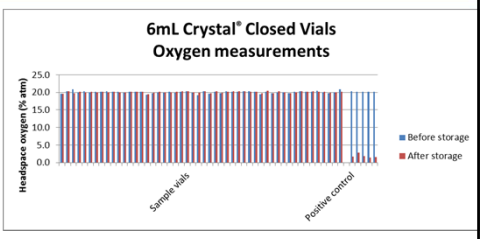
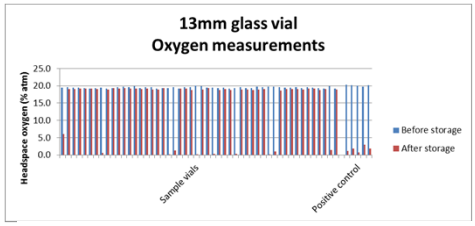
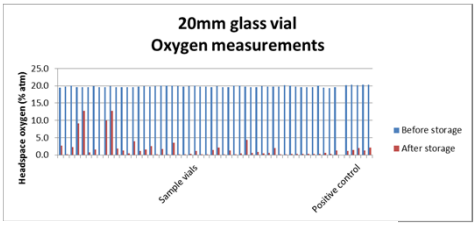
Crystal® Closed Vials vs. Stoppered glass vials





No CCI failures in Crystal® Closed Vials during cryo storage





High CCI failures in stoppered glass vials

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Future Studies…

Acknowledgements

- Josine Wilmer, Lighthouse Instruments
- Dave Markoch, Genesis Packaging Tech
- Brigitte Zuleger, IDT

Thank you for your attention!

For further discussion please come by the LIGHTHOUSE or Genesis Exhibits

Contact Information

Derek Duncan, PhD*
Director Product Line
Lighthouse Instruments
dduncan@lighthouseinstruments.com

Roger Asselta
Vice President and Senior Advisor
Genesis Technical Advisors
rasselta@gen-techno.com

*Presenter

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Past references

- *"Dry ice should not be used in shipping rubber-stoppered vials because the low temperatures can lead to shrinkage of the rubber with subsequent ingress of (unsterile) air and carbon dioxide."*

  Reference: "Recommendations on the Control and Monitoring of Storage and Transportation Temperatures of Medicinal Products", John Taylor (Medicines Control Agency UK), The Pharmaceutical Journal (Vol. 267), 28 July 2001, p.129

- **Effect of Low Temperatures on Parenteral Vial Seal Integrity**

  Asselta, R.; Smith, E.; Sunderland, W.; Trappler, E. Presented at the PDA Annual Meeting, Las Vegas, NV, 2007.

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Recent references

- **Identification of a Suitable Vial/Stopper Combination for Low Temperature Storage at -80C**

  Dr. Brigitte Zuleger, Bavarian Nordic, Presented at the PDA Parenteral Packaging Meeting, Berlin, 2012.

- **Container/Closure Integrity Testing and the Identification of a Suitable Vial/Stopper Combination for Low-Temperature Storage at -80 C**

  Brigitte Zuleger, Uwe Werner, Alexander Kort, Rene Glowienka, Engelbert Wehnes, Derek Duncan

  PDA J Pharm Sci and Tech 2012, 66 453-465

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